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Appositional enamel growth in molars of South African fossil hominids

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Abstract

Enamel is formed incrementally by the secretory activity of ameloblast cells. Variable stages of secretion result in the formation of structures known as cross striations along enamel prisms, for which experimental data demonstrate a correspondence with daily periods of secretion. Patterns of variation in this daily growth are important to understanding mechanisms of tooth formation and the development of enamel thickness. Transmitted light microscopy (TLM) of histological ground sections and scanning electron microscopy (SEM) of bulk specimens or their surface replicas are the usual methods for investigating cross striations. However, these methods pose some constraints on the study of these features in Plio-Pleistocene hominid enamel, the specimens of which may only rarely be sectioned for TLM or examined on only their most superficial surfaces for SEM. The recent development of portable confocal scanning optical microscopy (PCSOM) resolves some of the restrictions on fractured enamel surfaces, allowing the visualization of cross striations by direct examination. This technology has been applied here to the study of *Australopithecus africanus* and *Paranthropus robustus* hominid molars from the Plio-Pleistocene of South Africa. We hypothesize that these taxa have increased enamel appositional rates compared with modern humans, because despite having thicker enamelled molars (particularly *P. robustus*), the enamel crowns of these fossil taxa take an equivalent or reduced amount of time to form. Cross striations were measured in cuspal, lateral and cervical regions of the enamel crowns, and, within each region, the inner, middle and outer zones. Values obtained for *A. africanus* outer zones of the enamel crown are, in general, lower than those for *P. robustus*, indicating faster forming enamel in the latter, while both taxa show higher rates of enamel growth than modern humans and the African great apes. This demonstrates a relatively high degree of variability in the mechanisms underlying the development of enamel across taxa.

Key words appositional rate; enamel development; South African hominids.

Introduction

Cells known as ameloblasts, the main function of which is to secrete enamel matrix and are therefore not found in any other tissues, begin their secretory action in response to signals emanating from the odontoblasts (dentine-forming cells) (Karcher-Djuricic et al. 1985). Secretion begins at the sites of future dentine horn tips, proceeding then in the direction of the cervix (Jernvall & Thesleff, 2000). A process formed at the apical end of the cell, known as the Tomes process, is necessary for the correct development of structural units in mammalian enamel known as prisms, or rods, which are developed as by-products of secretory ameloblasts as they move from the dentine–enamel junction (DEJ) towards the outer enamel surface (OES) (Moss-Salentjin et al. 1997). In response to circadian (daily) rhythms, there is a variation in secretory activity by competent ameloblasts, which alternate between faster and slower secretory stages (Boyde, 1989). These differences in cellular behaviour between stages create structural features known as cross striations along individual enamel prisms, or rods (Boyde, 1964, 1989). Cross striations are commonly described as linear features running perpendicular to the main prism's path, or as varicosities (Dean, 1987, 2000; Boyde, 1989) (Fig. 1). Their appearance depends

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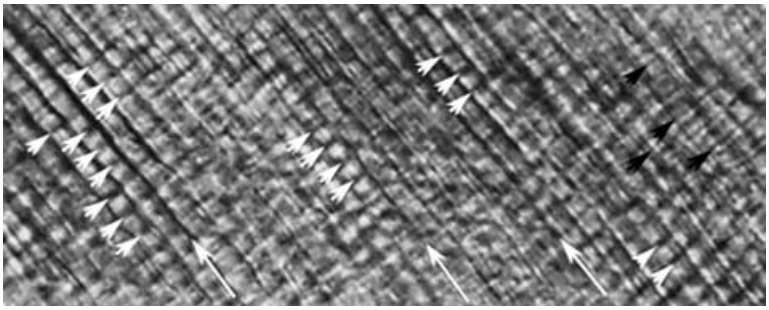

Fig. 1 Image taken using TLM in the middle region of the cuspal enamel of a modern human molar. Cross striations or daily cell secretions are indicated by small white arrows. Other enamel features that have a shorter periodic appearance than cross striations (Dean, 2000), known as intradian lines, are here indicated by small black arrows. Large white arrows indicate the main direction of prisms. The mean cross-striation length is about 4.5 μm in this image.

on the microscopic technique used, i.e. transmitted light microscopy (TLM) of ground sections, or scanning electron microscopy (SEM) of exposed internal enamel. There is structural and chemical evidence for the presence of cross striations. For instance, variations in CO_3 and Na concentrations have been detected in cross striations (Boyde, 1979; Driessens et al. 1984), and Simmelink & Nygaard (1982) reported differences in porosity at the sites where cross striations appear.

Confirmation of the daily appositional growth of ameloblasts in response to the cell's circadian rhythms is based on several experimental studies dating from the 1930s onward (Schour & Poncher, 1937; Massler & Schour, 1946; Boyde, 1964; Bromage, 1991; Smith, 2006). Secretory enamel cells also periodically experience a more marked physiological disruption, which results in the development of long-term markers known as striae of Retzius (Boyde, 1989). Striae appear as more pronounced lines passing at an oblique angle to the prisms (Dean, 1987). The number of cross striations between the Retzius lines is regarded as the cross striation repeat interval, or periodicity, which in modern humans ranges from 6 to 11 days (Dean, 2000) with a mean and modal value of 9 days (Dean & Reid, 2001). In the common chimpanzee (*Pan troglodytes*), values range from 6 to 8 days, with an average of 7 days (Reid et al. 1998; Smith, 2004).

Enamel thickness of the permanent dentition is an important character of the adult phenotype, and features prominently in taxonomic studies within the Hominoidea (Martin, 1985; Beynon & Wood, 1986; Grine & Martin, 1988; Macho & Thackeray, 1992; Macho, 1995; Schwartz, 2000; Smith et al. 2005). The development of enamel thickness is a function of the number of active secretory cells, daily secretion rates and the timing of preprogrammed cell death (apoptosis) (e.g. Grine & Martin, 1988; Beynon et al. 1991; Macho, 1995; Dean, 2000; Dean et al. 2001). Enamel thickness, however, can be achieved via a variety of developmental pathways over given time spans and thus cannot be said to be a homologous feature across primate taxa (Dean, 1998, 2000; Schwartz, 2000; Dean et al. 2001). Therefore, an understanding of the processes involved in the development of enamel thickness is more accurately interpreted at the cellular level where variation in daily secretion rates may show differences of its development (Dean, 2000).

Some trends in daily enamel growth of primates have been recognized. In general, daily rates, or cross striation spacing, tend to increase as cells move from the DEJ toward the OES, with a corresponding decrease in appositional growth rates from the cusp to the cervical end of the tooth (Beynon et al. 1991; Reid et al. 1998; Dean, 2000; Smith, 2004; Smith et al. 2004; Ramirez Rozzi & Lacruz, in press). Interestingly, daily secretion rates are consistent between different cusps, within each region of each cusp (i.e. inner, mid and outer) and between molar types in a large sample (n = 69) of ground sections of molars of *Pan troglodytes* (Smith, 2004).

Daily enamel growth rates have been recorded in only a few fossil hominoids (Dean et al. 1993, 2001; Beynon et al. 1998; Schwartz et al. 2003; Smith et al. 2003, 2004). These studies are based on small sample sizes, typically one or two specimens, because the histological sectioning of fossil hominid material is not commonly permitted. To address this problem, a portable confocal scanning optical microscope (PCSOM) has been developed, which provides optical 'sections', equivalent to those obtained by TLM, of enamel microanatomy from the naturally fractured surfaces of bulk specimens (Bromage et al. 2005, in press). The PCSOM allows observation and measurement of cross-striation intervals and therefore provides a unique opportunity to study variation in cellular behaviour during enamel growth on relatively large samples of fossil taxa.

We have applied this technology to the study of molars of the South African hominids *Australopithecus africanus* and *Paranthropus robustus.* Both fossil taxa are

characterized by thick-enamelled molars; *P. robustus* has 'hyper-thick' enamel whereas *A. africanus* is classified as having 'thick' enamel (Martin, 1985; Grine & Martin, 1988). Both taxa also have absolutely and relatively greater molar occlusal area than modern humans, and yet they appear to have formed their molars in similar or less time (Lacruz et al. in press). Given the differences in enamel thickness and the similarities in crown formation time between these taxa, our objective is to see if differences in molar thickness may in part be the result of different daily appositional rates.

There is only very limited data on cross-striation spacing (i.e. daily secretion rate) in Pliocene and early Pleistocene fossil hominid post-canine teeth; a premolar of *P. boisei* (Beynon & Dean, 1987) and a sample of unspecified *P. boisei* and early *Homo* molars from East Africa (Beynon & Wood, 1987) have been investigated to date. The distance between adjacent striae of Retzius was measured in *A. africanus* and East and South African *Paranthropus* molars (Beynon, 1992), and the results were then divided by a 7- and 8-day periodicity, because direct observation and measure of cross-striation spacing could not be obtained. Here we present values of cross-striation spacing, or daily appositional growth rates, in a relatively large sample of molars of *A. africanus* and *P. robustus* obtained by PCSOM. This information is compared with values recorded for modern humans and other primate taxa.

Materials and methods

We employed here a PCSOM described in detail by Bromage et al. (2003, 2005, in press). The operating principles are based on the Nipkow disc technique (Nipkow, 1884), which, in our case, employs a K2S-BIO confocal module configured to a custom stand, which facilitates vertical height adjustments. To image through long Z-height positions, the PCSOM is configured with 5×, 10×, 20× and 50× lenses, which provide working distances of 34, 19, 20 and 13 mm, respectively.

Image acquisition for the PCSOM is performed with a high-resolution 12-bit monochrome camera, which has a 2/3′ monochrome progressive scan interline CCD containing 1280 × 1024 pixels. Images are transferred in real-time to a notebook PC. PCSOM Illumination is provided by a 175-W Lambda LS xenon arc lamp, which transmits a flat and intense beam of light via a liquid light guide. The microscope returns image detail from a very thin optical plane at and immediately below the object surface (1–50 μm, depending upon specimen characteristics). To obtain two- or three-dimensional projections from a surface which is anything but perfectly flat, potential fields of view must be compiled from a through-series of captured images at all optical planes represented in the Z-axis. Images are imported into Syncroscopy Auto Montage (Syncroscopy Inc., Frederick, MD, USA), which montages only in-focus image content through a Z-series, permitting an even and fully representative image of either a pseudo-planar field of view or a three-dimensional reconstruction of surface or subsurface details.

An interesting feature of the disc design by Kino (1995) is the solution taken to suppress internal non-image-related reflections; the classic method of illuminating with polarized light to stop light reflections from within the optical system (e.g. from optical hardware within the body of the microscope), but not the useful light reflecting from the specimen and returning through the objective lens. Linear polarizing light filters and a single quarter-wave plate are employed for this purpose. The result is that all figures reported here using the PCSOM are, thus, confocal circularly polarized light images of enamel microstructure.

Measurements of cross-striation spacing in all teeth were taken using a measurement scheme partly based on Beynon et al. (1991), in which the enamel crown was divided into three areas corresponding to cuspal, lateral and cervical areas of each tooth (Fig. 2). At the same time, each of these areas was divided into inner, middle and outer zones, avoiding the enamel just above the cusp tips (typically characterized by 'gnarled' enamel). In order to include some specimens showing some degree of cuspal wear, our corresponding values for cuspal enamel were taken slightly more cervically than shown in the diagram of Beynon et al. (1991, their fig. 2C). Moreover, because it was difficult to obtain measurements near the DEJ, the values shown in inner enamel were taken no closer than 100–150 μm from the DEJ. These values may thus overestimate appositional growth in our inner enamel category when these are compared with modern taxa for which measurements were taken immediately adjacent to the DEJ.

Six molars of the South African taxa *A. africanus* derived from the dolomitic cave site of Sterkfontein (Member 4), and dated to about 2.5 Ma (Vrba, 1995), and seven molars of *P. robustus* from the sites of Swartkrans (Members 1–3) and Kromdraai B, dated at between 2.0 and 1.5 Ma (Brain, 1993; Thackeray et al.

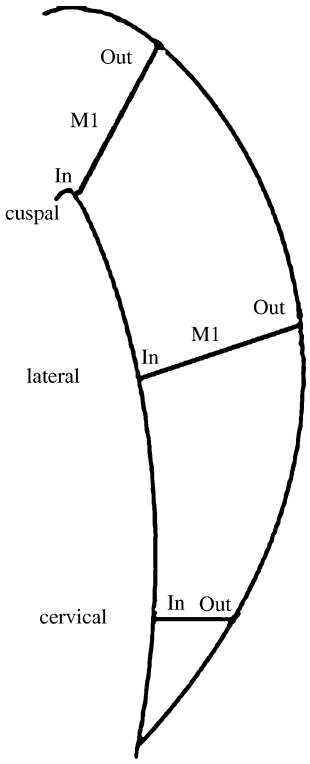

Fig. 2 Sketch modified from Beynon et al. (1991) indicating our measurement scheme of cross-striation spacing in fossil and modern samples used in this study.

2002), were used. Most teeth showed natural fractures developed *post mortem* and orientated in the occluso-cervical plane. The specimens were cleaned of any substances or matrix residue with acetone. The fractured surface of the tooth was placed approximately perpendicular to the optical axis of the PCSOM over which a drop of immersion oil and a cover slip were placed. To measure cross striations, the 50× lens was typically used with a 1 : 1 adapter; because the K2S-BIO produces a 2× magnification, this effectively results in 100× imaging conditions, providing a 190-μm field width. Measurements were taken in selected areas of the broken enamel that showed clear cross striations (Fig. 3), which in some cases were restricted to single fields of view for each division of the crown. Once these areas were identified, the distance of a minimum of 3–5 adjacent cross striations were measured in as many fields as possible. The values obtained for each group were divided by the number of cross striations measured (three or five), which yields a single representative value for cross striations at that site (Tables 1 and 2). To avoid possible discrepancies when comparing values obtained for the fossil taxa with published values for modern humans, a sample of ten modern human molars was studied using TLM of ground sections using the same measurement scheme as in the fossils. To assess differences between human and fossil hominid taxa, we employed the non-parametric Mann–Whitney *U* test.

Results

Table 1 show values obtained for *P. robustus* and *A. africanus*, indicating the number of groups of cross striations, which on average contained 3–5 individual cross striations that were measured in each division. Table 2 contains a summary of values of cross-striation spacing obtained for the modern human sample studied here, compared with the fossil taxa, and indicating the number of teeth used in each classification. It is noteworthy that the values obtained here for modern humans using our measurement scheme are very similar to values reported in Beynon et al. (1991), except in the cervical outer area for which this study shows greater values than those previously reported. Mann–Whitney *U* tests reveal significant differences between modern humans and the fossil taxa in all values along the crown. Significant differences between the two fossil groups were found only in the outer cuspal area.

Discussion

The terms 'appositional growth' or 'ameloblast secretion rates' used in this work refer to two-dimensional measurements of spacing between cross striations, a practice commonly employed in the literature. However, as aptly noted by Macho et al. (2003), this measurement does

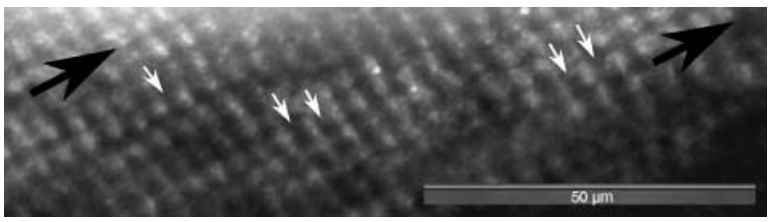

Fig. 3 Image taken with the PCSOM on the outer cervical enamel of the *Paranthropus robustus* specimen SK 55 from Swartkrans. Cross striations are marked with white arrows. Black arrows indicate prism direction. Image taken using a 50′ lens and 1 : 1 adapter. Scale bar = 50 μm.

Table 1 Measurements of cross-striation spacing (μm, mean ± SD) in *Paranthropus robustus* and *Australopithecus africanus* in different regions of the cusp

	Tooth	Face	Cu. Out	Cu. Mid.	Cu. Inn	Lat. Out	Lat. Mid.	Lat. Inn	Cerv. Out	Cerv. Inn
A. africanus										
Stw 11	UM3	D/B	6.25 ± 0.35 (10)	5.95 ± 0.30 (9)	?	6.07 ± 0.49 (8)	5.30 ± 0.34 (6)	?	3.95 ± 0.51 (6)	3.75 ± 0.33 (5)
Stw 37	UM3	B/L	6.75 ± 0.55 (9)	5.64 ± 0.30 (7)	4.07 ± 0.28 (5)	5.85 ± 0.23 (6)	5.20 ± 0.37 (8)	?	?	3.70 ± 0.21 (4)
Stw 284	UM2	M/L	6.85 ± 0.12 (7)	5.80 ± 0.32 (8)	4.25 ± 0.219 (6)	6.38 ± 0.28 (9)	5.45 ± 0.27 (7)	4.00 ± 0.16 (9)	4.70 ± 0.29 (5)	3.82 ± 0.12 (4)
Stw 217	UM1	D/B	6.90 ± 0.40 (5)	6.18 ± 0.23 (7)	?	6.40 ± 0.35 (5)	5.80 ± 0.37 (7)	4.10 ± 0.55 (6)	4.80 ± 0.20 (7)	3.70 ± 0.10 (5)
Stw 188	UM2	?	6.30 ± 0.31 (9)	?	?	6.18 ± 0.13 (7)	?	?	4.40 ± 0.45 (9)	?
Stw 96	LM3	M/B	?	?	?	5.8 ± 0.32 (9)	4.7 ± 0.21 (7)	?	?	?
P. robustus										
SK 55	LM2	D/B	7.55 ± 0.44 (11)	6.90 ± 0.14 (9)	?	7.05 ± 0.58 (9)	5.65 ± 0.12 (8)	?	4.62 ± 0.29 (10)	4.01 ± 0.17 (8)
Skx 21841	LM3	M/L	6.97 ± 0.33 (9)	5.64 ± 0.12 (8)	4.16 ± 0.14 (6)	6.45 ± 0.17 (10)	5.35 ± 0.31 (8)	4.18 ± 0.28 (5)	5.10 ± 0.40 (8)	3.98 ± 0.31 (5)
SK 875	frag		7.60 ± 0.44 (7)	6.20 ± 0.45 (6)	?	6.67 ± 0.32 (7)	5.27 ± 0.16 (9)	3.73 ± 0.21 (5)	4.75 ± 0.53 (7)	?
SK 35	LM2	M/L	6.90 ± 0.25 (8)	5.55 ± 0.18 (7)	?	6.58 ± 0.22 (9)	5.98 ± 0.23 (7)	?	4.40 ± 0.35 (10)	?
SK 37	LM2	D/B	?	?	?	6.10 ± 0.12 (7)	5.90 ± 0.23 (7)	?	4.95 ± 0.29 (8)	3.70 ± 0.12 (4)
SKW 4771	frag		7.16 ± 0.51 (7)	?	?	?	?	?	4.80 ± 0.33 (8)	?
TM 99	frag		?	?	?	?	?	?	5.19 ± 0.55 (6)	?

Tooth type and face studied are indicated. No measurements could be obtained for the areas where question marks are shown. The number in parentheses indicates the groups of cross striations measured in each region of the crown. In each group, 3–5 individual cross striations were measured.

Table 2 Results of cross-striation measurements on a sample of ten modern human molars compared with mean values (μm, ± SD) of *Paranthropus robustus* and *Australopithecus africanus* measured using the same scheme described in the text. Modern humans show much lower rates than both hominid taxa

	Cusp. out	Cusp. mid	Cusp. Inn	Lat. out	Lat. mid.	Lat. Inn	Cerv. out	Cerv. inn
H. sapiens	5.20 ± 0.58 (10)	4.50 ± 0.55 (10)	2.80 ± 0.43 (10)	4.80 ± 0.67 (10)	4.30 ± 0.5 (10)	2.70 ± 0.42 (10)	3.60 ± 0.44 (9)	2.60 ± 0.44 (10)
P. robustus	7.25 ± 0 .44 (5)	6.12 ± 0.56 (4)	4.16 (1)	6.59 ± 0.28 (5)	5.63 ± 0.27 (5)	3.95 ± 0.25 (2)	4.83 ± 0.26 (7)	3.89 ± 0.02 (3)
A. africanus	6.62 ± 0.55 (5)	5.80 ± 0.30 (4)	4.18 ± 0.75 (2)	6.11 ± 0.37 (6)	5.25 ± 0.39 (5)	4.20 (2)	4.46 ± 0.22 (4)	3.74 ± 0.29 (4)

The number in parentheses is the number of teeth included in each category for each taxon.

not reflect the 'true' secretion rate of ameloblasts as the diameter of prisms is not known, and this information is vital to assess the real volume of matrix secretion. However, as pointed out by Dean (2004), it appears that prism diameter does not increase much from inner to outer enamel, and that, in general, values remain close to 5 μm (see Dean, 2004, and references therein).

The study of naturally fractured enamel has some limitations as compared with investigations based on histological sections where prisms may be followed entirely from the DEJ to the OES (Beynon et al. 1998; Dean, 1998, 2004; Dean et al. 2001). In natural fractures, prisms disappear suddenly and abrupt changes in surface topography from micrometres to millimetres occur between adjacent fields, making it difficult to follow prisms for any great length. Even then, cross striations may not always be visible in the areas where prisms remain straight, probably due to diagenetic processes. However, a substantial improvement of PCSOM over conventional light microscopy implies not having to produce a thin section as a prerequisite for excellent optical microscopy of rare and unique specimens.

The results presented here constitute a significant advance in our understanding of the variation in early hominid enamel growth mechanisms. They indicate that *A. africanus* and *P. robustus* show a similar trend of increase in growth from the inner to outer enamel areas, and a decrease from cuspal to cervical enamel (Tables 1 and 2). The Sterkfontein sample of *A. africanus*, which has thick enamel (but less so than *P. robustus*; Grine & Martin, 1988; Macho & Thackeray, 1992), has a slightly smaller cross-striation spacing in the outer enamel than *P. robustus*. The values reported in Beynon & Wood (1987) for *P. boisei* molars in mid cuspal enamel are greater than the mean obtained here for the two South African hominids in the same tooth region, although the *P. boisei* value falls within the range obtained here. The South African taxa show higher daily rate values than those reported for early *Homo* (Beynon & Wood, 1987). Beynon (1992) reported outer cuspal values in *P. boisei* molars that ranged from 6.9 to 7.9 μm by measuring the distance between striae and assuming a 7- or 8-day periodicity. These values are similar to those reported here for *P. robustus*. For *A. africanus*, Beynon (1992) observed values in lateral and cervical enamel that are similar to those obtained in this study for the same regions. However, some discrepancies are noticeable between his values for *P. robustus* and our values. The values recorded in cuspal enamel for *P. robustus* in Beynon (1992) are lower than the values reported here. In fact, Beynon (1992) recorded nearly constant appositional rates, ranging from about 5 to 6 μm, from cuspal to cervical enamel, which differs from our findings (Tables 1 and 2).

When daily appositional rates are compared between fossil hominids and modern humans (Risnes, 1986; Beynon et al. 1991; Dean, 1998; this study, Table 2), the extinct hominids show greater values for each given region of the cusp. In the mid and outer cuspal region, for example, the fossil taxa show values ranging from 20 to 35% higher than in *H. sapiens*. Comparing data available for the great apes (Beynon et al. 1991; Dean, 1998; Reid et al. 1998; Smith, 2004), we also observe that Plio-Pleistocene hominid appositional rates exceed those observed for apes in each crown division. The highest values recorded by these studies include the mean of outer cuspal enamel of *Gorilla* (6.1 μm, Beynon et al. 1991) and a single value recorded in a molar of *H. sapiens* (6.4 μm, Dean, 1998). Both values fall below the appositional rate means of the fossil taxa in the same crown region (Table 2). It is noteworthy that none of the extant primate taxa used in these studies has greater enamel thickness than either *A. africanus* or *P. robustus*.

Conclusion

Using a novel PCSOM, this study has investigated variation in daily appositional rates in broken molar enamel surfaces of *P. robustus* and *A. africanus*. This is the first study of this nature to observe variation in daily growth rates in cuspal, lateral and cervical enamel of a relatively large sample of molars of Plio-Pleistocene hominids. Given that *P. robustus* and *A. africanus* show differences in enamel thickness, but appear to have formed their molar crowns in similar time, it was expected that some differences in appositional growth would be found. In general, *P. robustus* shows higher outer values than *A. africanus*, and both fossil taxa have higher values than modern *H. sapiens* and the great apes. High daily secretion rates appear to be one of the mechanisms employed by both megadont fossil hominid taxa studied here to form thick enamelled molars.

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